



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : A61K 37/00, 37/02, 37/26		A1	(11) International Publication Number: WO 94/08603 (43) International Publication Date: 28 April 1994 (28.04.94)
<p>(21) International Application Number: PCT/US93/09915</p> <p>(22) International Filing Date: 15 October 1993 (15.10.93)</p> <p>(30) Priority data: 07/962,956 16 October 1992 (16.10.92) US</p> <p>(60) Parent Application or Grant (63) Related by Continuation US Filed on 07/962,956 (CIP) 16 October 1992 (16.10.92)</p> <p>(71) Applicant (<i>for all designated States except US</i>): SMITH-KLINE BEECHAM CORPORATION [US/US]; Corporate Intellectual Property, UW2220, 709 Swedeland Road, P.O. Box 1539, King of Prussia, PA 19406-0939 (US).</p>		<p>(72) Inventor; and (75) Inventor/Applicant (<i>for US only</i>) : CONSTANTINIDES, Panayiotis, Pericleous [US/US]; 1027 North Valley Forge Road, #305, Devon, PA 19333 (US).</p> <p>(74) Agents: DINNER, Dara, L. et al.; SmithKline Beecham Corporation, Corporate Intellectual Property, UW2220, 709 Swedeland Road, P.O. Box 1539, King of Prussia, PA 19406-0939 (US).</p> <p>(81) Designated States: JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).</p> <p>Published <i>With international search report.</i></p>	
<p>(54) Title: COMPOSITIONS</p> <p>(57) Abstract</p> <p>Compositions are described comprising an interesterified triglyceride; a low HLB surfactant which is a medium- or a long-chain fatty acyl mono- and/or diglyceride, a sorbitan long-chain fatty acid ester or mixtures thereof; a high HLB surfactant; an aqueous hydrophilic phase; and a water-soluble therapeutic agent which on admixing form a stable, self-emulsifying, water-in-oil (w/o) microemulsion.</p>			

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FR	France	MR	Mauritania
AU	Australia	GA	Gabon	MW	Malawi
BB	Barbados	GB	United Kingdom	NE	Niger
BE	Belgium	GN	Guinea	NL	Netherlands
BF	Burkina Faso	GR	Greece	NO	Norway
BC	Bulgaria	HU	Hungary	NZ	New Zealand
BJ	Benin	IE	Ireland	PL	Poland
BR	Brazil	IT	Italy	PT	Portugal
BY	Belarus	JP	Japan	RO	Romania
CA	Canada	KP	Democratic People's Republic of Korea	RU	Russian Federation
CF	Central African Republic	KR	Republic of Korea	SD	Sudan
CG	Congo	KZ	Kazakhstan	SE	Sweden
CH	Switzerland	LJ	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovak Republic
CM	Cameroon	LU	Luxembourg	SN	Senegal
CN	China	LV	Latvia	TD	Chad
CS	Czechoslovakia	MC	Monaco	TG	Togo
CZ	Czech Republic	MG	Madagascar	UA	Ukraine
DE	Germany	ML	Mali	US	United States of America
DK	Denmark	MN	Mongolia	UZ	Uzbekistan
ES	Spain			VN	Viet Nam
FI	Finland				

COMPOSITIONS

5

FIELD OF THE INVENTION

This invention relates to pharmaceutical compositions in the form of water-in-oil (w/o) self-emulsifying microemulsions, processes for their preparation and their use.

10 BACKGROUND OF THE INVENTION

Microemulsions can be defined in general as thermodynamically stable, isotropically clear dispersions of two immiscible liquids stabilized by interfacial films of surface-active molecules. The formation of microemulsions usually involves a combination of three to five components, namely, an oil, water, a surfactant, a cosurfactant and an electrolyte. The tendency to form either a water-in-oil (w/o) or an oil-in-water (o/w) microemulsion is influenced by the properties of the oil and the surfactant. Surfactants are conveniently classified on an empirical scale known as the hydrophilic-lipophilic balance (HLB) which runs from 1 to 20. In general, (w/o) microemulsions are formed using surfactants (or emulsifiers) which have an HLB value in the range of about 15 3 to 6 while (o/w) microemulsions are formed using surfactants which have an HLB value in the range of about 8 to 18. It has long been recognized that low interfacial tension contributes to the thermodynamic stability of microemulsions. To achieve this, the surfactant should preferably exhibit low solubility in both the oil and water phases, and be preferentially absorbed at the water-oil interface with concomitant lowering of interfacial 20 tension. When interfacial tension is less than 2×10^{-2} dyn/cm, a stable microemulsion can form. General reviews of microemulsions are provided by Bhargava *et al.*, Pharm. Tech., 46-53, March 1987 and Kahlweit, Science, 240, 617-621, 1988.

Microemulsions are typically substantially non-opaque, that is they are transparent 30 or opalescent when viewed by optical microscopic means. In the undisturbed state, they are optically isotropic (non-birefringent) when examined under polarized light. The dispersed phase typically comprises particles or droplets which are normally between 5 and 200 nm in size and this gives rise to their optical transparency. These particles may be spherical although other structures are feasible.

35

The role of the cosurfactant, usually a short-chain alcohol, is to increase the interfacial fluidity by penetrating the surfactant film and consequently creating a disordered film due to the void space among surfactant molecules. The use of a cosurfactant in

microemulsions is however optional and alcohol-free self-emulsifying emulsions and microemulsions have been described in the literature (see for instance, Pouton et al., Int. Journal of Pharmaceutics, 27, 335-348, 1985 and Osborne et al., J. Disp. Sci. Tech., 9, 415-423, 1988).

5

There are many advantages to the use of a microemulsion over a conventional emulsion (or macroemulsion) for drug transport (delivery). Microemulsions form spontaneously, without the need for a high input of energy and are therefore easy to prepare and scale up for commercial applications; they have thermodynamic stability due 10 to their small particle size and therefore have a long shelf life; they have an isotropically clear appearance so that they may be monitored by spectroscopic means; they have a relatively low viscosity and are therefore easy to transport and mix; they have a large interfacial area which accelerates surface reactions; they have a low interfacial tension which permits flexible and high penetrating power and, lastly, they offer the possibility of 15 improved drug solubilization and protection against enzymatic hydrolysis. In addition, microemulsions may undergo phase inversion upon addition of an excess of the dispersed phase or in response to a temperature change and this is a property of these systems that can affect drug release from microemulsions both *in vitro* and *in vivo*. The reasons for this improved drug delivery are not however well understood.

20

The use of lipid-based microemulsions to enhance the bioavailability of different drugs, including peptides, has already been proposed. Thus, GB 2 222 770-A (Sandoz Ltd) describes microemulsions and corresponding microemulsion "pre-concentrates" for use with the highly hydrophobic cyclosporin peptides. Thus, a suitable pre-concentrate 25 comprises 1,2-propylene glycol as the hydrophilic component, a caprylic-capric acid triglyceride as the lipophilic component and a mixture of a polyoxyethylene glycolated hydrogenated castor oil and glycerin monooleate (ratio 11:1) as the surfactant-cosurfactant. Such formulations may then be diluted with water, to give oil-in-water rather than water-in-oil microemulsions.

30

GB 2 098 865A (Sandoz Ltd) describes topical compositions in the form of microemulsions comprising a water-immiscible organic solvent, an emulsifier, a co-emulsifier, water and a (non-peptide) therapeutic agent. These formulations are said to have improved skin penetrating properties. Suitable organic solvents include mono- or 35 diesters of glycerol with a (C₆-22) carboxylic acid, such as glyceryl caprylate (which may also act as a co-emulsifier).

US 4 712 239 (Muller *et al.*) describes multi-component systems for pharmaceutical use comprising an oil, a nonionic surfactant with an HLB value above 8 and a co-surfactant which is a partial ether or ester of a polyhydroxyl alcohol and a (C₆₋₂₂) fatty alcohol or acid, which components form a "single phase" on mixing. The special properties of the system are attributed to the particular blend of surfactant and co-surfactant selected. An aqueous phase is an optional extra and the therapeutic agent may be lipophilic or hydrophilic. Such systems are said to give enhanced transdermal delivery characteristics. Amongst the examples provided, one (example 1, formulation I) has PEG (20 EO)-oleic acid glycerol partial esters (40%), caprylic-capric acid glycerol partial esters (42% monoglyceride, 24%), medium-chain triglycerides (16%) and water (20%).

GB 1 171 125 (Glaxo Laboratories Ltd.) describes microemulsions comprising a hydrophilic oil, a blend of low and high HLB surfactants and an aqueous phase, for use as injectable preparations. In particular, example 15 thereof contains in the lipophilic phase a mixture of coconut oil and sorbitan monooleate. The patent is concerned with improved formulations and is silent on bioavailability.

WO 88/00059 (Engström *et al.*, and the corresponding paper, *J. Dispersion Sci. Technol.*, 11, 479, 1990) discloses controlled release compositions for biologically active materials comprising an "L2-phase" and containing an unsaturated (C₁₆₋₂₂)-fatty acid monoglyceride and an unsaturated (C₁₆₋₂₂)-fatty acid triglyceride, in a ratio of from 1:1 to 3:1, and a polar liquid such as water. Such an unsaturated (C₁₆₋₂₂)-fatty acid monoglyceride is a low HLB surfactant. There is, however, no mention of the additional inclusion of a high HLB surfactant. The existence of an L2 phase had previously been described for a water/monocaprylin/tricaprylin system by Friberg *et al.*, *J. Amer. Oil Chem. Soc.*, 47, 149, 1970. Again, there is no mention of the additional inclusion of a high HLB surfactant.

Surprisingly it has now been found that useful drug delivery characteristics may also be obtained using (w/o) microemulsions in which the triglyceride is chemically modified to comprise a mixture of medium- and long-chain fatty acyl moieties.

SUMMARY OF THE INVENTION

Accordingly, the present invention provides a pharmaceutical composition comprising:

- (a) a lipophilic phase having an oil which comprises an interesterified triglyceride and a low HLB surfactant wherein the low HLB surfactant is selected from a medium fatty acyl monoglyceride, a long chain fatty acyl mono-glyceride, a long-chain fatty

- acyl di-glyceride, a medium chain fatty acyl di-glyceride, a sorbitan long-chain fatty acid ester or mixtures thereof;
- (b) a high HLB surfactant;
- (c) an aqueous hydrophilic phase; and
- 5 (d) a water-soluble therapeutic agent. The pharmaceutical composition on admixing forms a stable, self-emulsifying, water-in-oil (w/o) microemulsion.

DETAILED DESCRIPTION OF THE FIGURES

- Figure 1 Illustrates a Pseudo-Ternary Phase Diagram reading of a Microemulsion System containing the mixture of the oil and the low HLB surfactant, at a fixed ratio X, a hydrophilic (aqueous) phase and a high HLB surfactant.
- 10 Figure 2 Illustrates a Pseudo Ternary Phase Diagram of the W/O Microemulsion Existence Field in the System comprising CAPTEX 810A/ARLACEL 186 (in the ratio 3:1); a high HLB surfactant (TWEEN 80); and saline.
- 15 Figure 3 Illustrates a Pseudo Ternary Phase Diagram of the W/O Microemulsion Existence Field in the System comprising CAPTEX 810A /CAPMUL MCM (ratio 3:1), a high HLB surfactant TWEEN 80 and saline.
- Figure 4 Illustrates a Pseudo Ternary Phase Diagram of the W/O Microemulsion Existence Field in the System comprising CAPTEX 910A/ARLACEL 186 (ratio 3:1), a high HLB surfactant TWEEN 80 and saline.
- 20 Figure 5 Illustrates a Pseudo Ternary Phase Diagram of the W/O Microemulsion Existence Field in the System comprising Captex 910A/CAPMUL MCM (ratio 3:1), a high HLB surfactant TWEEN 80 and saline.
- Figure 6 Illustrates a Pseudo Ternary Phase Diagram of the W/O Microemulsion Existence Field in the System comprising CAPTEX 910B/ARLACEL 186 (ratio 3:1), a high HLB surfactant TWEEN 80 and saline.
- 25 Figure 7 Illustrates a Pseudo Ternary Phase Diagram of the W/O Microemulsion Existence Field in the System comprising Captex 910B/CAPMUL MCM (ratio 3:1), a high HLB surfactant TWEEN 80 and saline.
- 30 Figure 8 Illustrates a Pseudo Ternary Phase Diagram of the W/O Microemulsion Existence Field in the System comprising CAPTEX 910C/ARLACEL 186 (ratio 3:1), a high HLB surfactant TWEEN 80 and saline.
- Figure 9 Illustrates a Pseudo Ternary Phase Diagram of the W/O Microemulsion Existence Field in the System comprising Captex 910C/CAPMUL MCM (ratio 3:1), a high HLB surfactant TWEEN 80 and saline.
- 35

DETAILED DESCRIPTION OF THE INVENTION

As noted above the instant invention comprises a pharmaceutical composition which has

- 5 (a) a lipophilic phase having an oil which comprises an interesterified triglyceride and a low HLB surfactant wherein the low HLB surfactant is selected from a medium fatty acyl monoglyceride, a long chain fatty acyl mono-glyceride, a long-chain fatty acyl di-glyceride, a medium chain fatty acyl di-glyceride, a sorbitan long-chain fatty acid ester or mixtures thereof;
- 10 (b) a high HLB surfactant;
- 10 (c) an aqueous hydrophilic phase; and
- 10 (d) a water-soluble therapeutic agent;
- which on admixing form stable, self-emulsifying, water-in-oil (w/o) microemulsions.

15 Earlier work in this area disclosed that useful (w/o) microemulsions may be prepared using a medium-chain fatty acyl triglyceride and a low HLB surfactant which is a medium-chain fatty acyl mono- or di-glyceride or a mixture thereof (Constantinides, P., WO93/02664, published 18 February 1993) or a long-chain fatty acyl triglyceride oil and a low HLB surfactant which is a long-chain fatty acyl mono- or di-glyceride or a mixture thereof or a sorbitan long-chain fatty acyl ester (Constantinides, P., WO93/02665, 20 published 18 February 1993). The use of an interesterified triglyceride, a physical admixture of medium- and long-chain fatty acyl triglycerides in combination with a low HLB surfactant has also surprising been found to produce stable, water-in-oil (w/o) self-emulsifying microemulsions.

25 The term "medium-chain fatty acyl" as used herein refers to a fatty acyl moiety having from 6 to 12, preferably 8 to 10 carbon atoms which may be branched or unbranched, preferably unbranched and which may be optionally substituted.

30 The term "long-chain fatty acyl" as used herein refers to a fatty acyl moiety which may be saturated, mono-unsaturated or poly-unsaturated, having from 14 to 22, preferably 14 to 18, carbon atoms which may be branched or unbranched, preferably unbranched, and which may be optionally substituted.

35 The term "interesterified triglyceride" as used herein, refers to a triglyceride which is formed synthetically from reacting together a physical admixture of medium- and long-chain fatty acyl triglycerides to form new synthetic triglycerides in which each triglyceride has a mixture of medium- and long-chain fatty acyl moieties. Suitable such triglycerides are readily available from commercial suppliers, having already found use in the food

industry. It will be appreciated that naturally occurring oils such as coconut or palm oil comprise triglycerides formed randomly from a mixture of a number of different medium- and long-chain fatty acids and can therefore be distinguished over triglycerides that are enriched in long-chain fatty acyl chains useful in the present invention.

5

Suitable interesterified triglycerides for use in the present invention include those in which the medium-chain fatty acyl moieties comprise caprylic and/or capric acids and the long-chain fatty acyl moieties comprise oleic and/or linoleic acids. Preferably, the medium-chain fatty acyl moieties comprise from about 30 to 90%, more preferably from 10 about 40 to 80%, most preferably from about 50 to 80% by weight of the fatty acyl moieties. Preferably, the long-chain fatty acyl moieties comprise from about 10 to 50%, more preferably from about 10 to 40%, most preferably about 10 to 30% by weight of the fatty acyl moieties. Preferably, the medium-chain fatty acyl moieties comprise a majority of caprylic acid moieties. Suitably, the ratio of caprylic acid and capric acid moieties is in 15 the range of from about 1:1 to 9:1, more suitably about 1:1 to 3:1.

Suitable triglycerides include the products available from Karisham Lipid Specialties, Columbus OH under the trade marks CAPTEX 810A, B, C and D and CAPTEX 910A, B, C and D which are described as interesterified medium-chain 20 triglycerides and high linoleic or oleic vegetable oil and comprise respectively mixtures of caprylic/capric and linoleic acids and caprylic/capric and oleic acids and essential fatty acids, as detailed in the following table (manufacturer's data, fatty acid % by weight):

Fatty Acid Composition, % W/W			
Product	Medium-chain	Long-chain	Other
810A	78	14	6
810B	58	27	15
810C	44	37	19
810D	32	45	45
910A	80	15 + 1*	4
910B	60	32 + 2*	6
910C	50	41 + 3*	6
910D	33	56 + 4*	7

*linoleic acid

Suitable low HLB surfactants for use in the present invention include fatty acyl mono- and di-glycerides, as well as mixtures thereof, and may also include a small amount by weight (less than 5, preferably less than 2% by weight) of free fatty acid. The mono- and di-glycerides may each include blends of different fatty acid mono- and di-glycerides.

Suitable medium chain fatty acid mono- and di-glycerides are formed from caprylic and capric acids. Suitable blends comprise from about 50 to 100% caprylic acid and from about 0 to 50% capric acid mono and/or diglycerides. Suitable commercial sources of these include the products available under the trade name CAPMUL (Karlsham Lipid Specialties, Columbus OH), for instance the products CAPMUL MCM which comprises monoglycerides (77.4%), diglycerides (21%) and free glycerol (1.6%), with a fatty acid composition of caproic acid (3.2%), caprylic acid (66.8%), capric acid (29.6%), lauric acid (0.3%) and palmitic acid (0.1%) and CAPMUL C8 which has monoglycerides (70 - 90%), diglycerides (10 - 30%) and free glycerol (2- 4%), with a fatty acid composition which comprises at least 98% caprylic acid. The ester composition of capmul products provided by the manufacturer is expressed as oleates (C18); actual C8/C10 monoester content is about 45% of each. Another suitable blend of mainly caprylic acid mono- and di-glycerides is the product Imwitor 308, from Hüls America, Piscataway NJ, which is similar to CAPMUL C8.

Suitable long-chain fatty acid monoglycerides include glycerol monooleate, glycerol monopalmitate and glycerol monostearate. Suitable commercially available examples of such include the products available under the trade names MYVEROL, such as MYVEROL 18-92 and 18-99, MYVATEX and MYVAPLEX, respectively, from Eastman Kodak Chemicals, Rochester, New York. A further useful long-chain fatty acid monoglyceride-containing product is ARLACEL 186 (available from ICI Americas Inc.) which includes, in addition to glycerol monooleate, propylene glycol (10%). The main fatty acids of MYVEROL 18-99 are oleic acid (61%), linoleic acid (21%), linolenic acid (9%) and palmitic acid (4%). Suitably in such long-chain monoglycerides, the major fatty acid component is a C₁₈-saturated, mono-unsaturated or polyunsaturated fatty acid, preferably a C₁₈-mono-unsaturated or polyunsaturated fatty acid. In addition, diacetylated and disuccinylated versions of the monoglycerides such as the product available under the trade name MYVATEX SMG are also useful.

35

Further suitable low HLB surfactants for use in the present invention include sorbitan long-chain fatty acid esters such as sorbitan monooleate, available commercially

under the trade names SPAN 80 and ARLACEL 80 and sorbitan sesquioleate, available commercially under the trade names SPAN 83 and ARLACEL 83.

5 Of these various low HLB surfactants hereinbefore described, the use of caprylic acid/capric acid mono- and di-glycerides, in particular, caprylic and capric acid monoglycerides, is especially preferred.

Suitably, the low HLB surfactant will have an HLB value in the range of about 2.5 to 6. The HLB values of the products CAPMUL MCM, MYVEROL 18-99, ARLACEL 10 10, ARLACEL 83 and ARLACEL 186 are respectively about 5.5 to 6, 3.7, 4.3, 3.7 and 2.8.

Suitable high HLB surfactants for use in the present invention include non-ionic surfactants, such as

15 (a) polyoxyethylene fatty acid esters, for example polyoxyethylene stearic acid esters of the type available under the trade name MYRJ (ICI Americas, Inc.), for instance the product MYRJ 52 (a polyoxyethylene 40 stearate);

20 (b) polyoxyethylen-sorbitan fatty acid esters (polysorbates), for example the mono- and tri-lauryl, palmityl, stearyl and oleyl esters, for instance the polyoxyethylene sorbitan monooleates available under the trade name of TWEEN (ICI Americas Inc.), such as TWEEN 20, 21, 40, 60, 61, 65, 80, 81 and 85, of which class TWEEN 80 is especially preferred;

(c) polyoxyethylene glycol long-chain alkyl ethers, such as polyoxyethylated glycol lauryl ether; and

25 (d) polyoxyethylene glycol long-chain alkyl esters, such as PEG-monostearate.

For use herein, the high HLB surfactant preferably has an HLB value in the range of 13 to 20.

30 Suitably, the blend of low and high HLB surfactants will have an HLB value in the range of from about 7 to about 15.

The term "therapeutic agent" (hereinafter also referred to as "drug") as used herein, refers to any compound which has biological activity, is soluble in the hydrophilic phase 35 and has an HLB value of at least that of the high HLB surfactant used in the formulation, to ensure that the drug is preferentially dissolved in the hydrophilic rather than the lipophilic phase. This includes both peptides and non-peptides. Suitable peptides include not only small peptides but also larger peptides/polypeptides and proteins. Suitable such

peptides preferably have a molecular weight from about 100 to 10,000, more preferably from about 100 to about 6,000. Especially preferred are peptides having from 2 to 35 amino acid moieties. Higher molecular weight peptides, even those with a molecular weight of above 10,000, up to about 50,000, may also be accommodated in microemulsions 5 of the present invention.

Suitable small peptides have from about 2 to about 10, more preferably from about 2 to about 6 amino acid moieties. Preferred small peptides include the fibrinogen receptor antagonists (RGD containing peptides) which are tetrapeptides with an average molecular 10 weight of about 600. These peptide antagonists are highly potent platelet aggregation inhibitors at plasma levels as low as 1 pmol/ml. Preferred fibrinogen antagonists include the peptide cyclo(S,S)-N^a-acetyl-Cys-(N^a-methyl)Arg-Gly-Asp-Pen-NH₂ (Ali *et al.*, EP 0 341 915, whose disclosure is herein incorporated by reference in its entirety) and the peptide cyclo(S,S)-(2-mercaptophenylamido (EP 0 423 212, whose disclosure is herein incorporated by 15 reference in its entirety). Other fibrinogen antagonists useful in the present invention are those peptides disclosed by Pierschbacher *et al.*, WO 89/05150 (US/88/04403); Marguerie, EP 0 275 748; Adams *et al.*, U.S. 4,857,508; Zimmerman *et al.*, U.S. 4,683,291; Nutt *et al.*, EP 0 410 537, EP 0 410 539, EP 0 410 540, EP 0 410 541, EP 0 20 410 767, EP 0 410 833, EP 0 422 937 and EP 0 422 938; Ali *et al.*, EP 0 372 486; Ohba *et al.*, WO 90/02751 (PCT/JP89/00926); Klein *et al.*, U.S. 4,952,562; Scarborough *et al.*, WO 90/15620 (PCT/US90/03417); Ali *et al.*, PCT/US90/06514 and PCT/US92/00999; the peptide-like compounds disclosed by Ali *et al.*, EP 0 381 033 and EP 0 384 362; and the RGD peptide cyclo-N^a-acetyl-Cys-Asn-Dtc-Amf-Gly-Asp-Cys-OH 25 (in which Dtc is 4,4'-dimethylthiazolidine-5-carboxylic acid and Amf is 4-aminomethylphenylalanine).

The RGD peptide may be usefully included in the microemulsion formulation in an amount up to about 600mg/g of the hydrophilic phase or from 0.1 to 60 mg/g of the 30 formulation.

Other peptides useful in the present invention include, but are not limited to, other RGD containing peptides such as those disclosed by Momany, US 4,411,890 and US 4,410,513; Bowers *et al.*, US 4,880,778, US 4,880,777, US 4,839,344; and WO 35 89/10933 (PCT/US89/01829); the peptide Ala-His-D-Nal-Ala-Trp-D-Phe-Lys-NH₂ (in which Nal represents β -naphthylalanine) and the peptides disclosed by Momany, US 4,228,158, US 4,228,157, US 4,228,156, US 4,228,155, US 4,226,857, US 4,224,316, US 4,223,021, US 4,223,020, US 4,223,019 and US 4,410,512.

Other suitable peptides include hexapeptides such as the growth hormone releasing peptide (GHRP) His-D-Trp-Ala-Trp-D-Phe-Lys-NH₂, (Momany, US 4,411,890) and related analogs or homologs thereof, such as but not limited to, His-D-Phe-Ala-D-Phe-Lys-Gln-Gly-NH₂, Hong et al., USSN 07/951500 the disclosure of 5 which are herein incorporated by reference in their entirety). This may usefully be included in an amount up to about 250mg/g of the hydrophilic phase or from 0.1 to 25mg/g of the formulation.

Suitable larger polypeptides and proteins for use in microemulsions of the present 10 invention include insulin, calcitonin, elcatonin, calcitonin-gene related peptide and porcine somatostatin as well as analogs and homologs thereof. Other suitable larger polypeptides include those disclosed by Pierschbacher et al., US 4,589,881 (>30 residues); Bittle et al., US 4,544,500 (20-30 residues); and Dimarchi et al., EP 0 204 480 (>34 residues).

15 Other type of compounds useful in the present invention include analogs or homologs of LHRH which display potent LH releasing activity or inhibit the activity of LHRH; analogs or homologs of HP5 which possesses hematopoetic activity; analogs or homologs of endothelin which possess hypotensive activity; analogs or homologs of enkephalin which have antinociceptive activity; analogs or homologs of chloreccystokininin; 20 analogs or homologs of cyclosporin A which have immunosuppressive activity; analogs or homologs of atrial natriuretic factor; peptidergic antineoplastic agents; analogs or homologs of gastrin releasing peptide; analogs or homologs of somatostatin; gastrin antagonists; bradykinin antagonists; neurotensin antagonists; bombesin antagonists; oxytocin agonists and antagonists; vasopressin agonists and antagonists; hirudin analogs 25 and homologs; analogs and homologs of the cytoprotective peptide-cyclolinopeptide; alpha MSH analogs; analogs, and homologs of MSH releasing factor (Pro-Leu-Gly-NH₂); peptides which inhibit collagenase; peptides which inhibit elastase, peptides which inhibit renin; peptides which inhibit HIV protease; peptides which inhibit angiotensin converting enzyme; peptides which inhibit chymases and tryptases and peptides which 30 inhibit blood coagulation enzymes.

Other suitable drugs include non-peptide therapeutic agents such as antibiotics, antimicrobial agents, antineoplastic agents, cardiovascular and renal agents, antiinflammatory, immunosuppressive and immunostimulatory agents and CNS agents.

35

Preferably, the drug is a peptide such as a fibrinogen receptor antagonist peptide (an RGD peptide), GHRP (His-D-Trp-Ala-Trp-D-Phe-Lys-NH₂), a vasopressin, a calcitonin or an insulin, more preferably the fibrinogen receptor antagonist peptides

cyclo(S,S)-N^a-acetyl-Cys-(N^a-methyl)Arg-Gly-Asp-Pen-NH₂ or cyclo(S,S)-(2-mercaptop)benzoyl-(N^a-methyl)Arg-Gly-Asp-(2-mercaptop)phenylamide or GHRP.

In a preferred aspect, the present invention provides compositions in the form of
5 microemulsions comprising a peptide which may be orally administered and which will retain biological activity, thereby overcoming the disadvantages of earlier formulations in which the bioavailability of the peptide has been less than satisfactory. In particular, the present invention provides compositions which by their nature permit the preparation and administration of a peptide in sufficiently high concentration to allow not only convenient
10 oral administration but also adequate bioavailability of the peptide.

For a water-soluble drug, the degree of incorporation into (w/o) compositions of the present invention is limited only by its solubility in the hydrophilic phase. The ionic strength and pH (within the range 3 to 10) may be adjusted to aid dissolution, without
15 compromising the integrity of the composition.

The aqueous hydrophilic phase suitably comprises water or an isotonic saline solution and may also include a pharmaceutically acceptable solvent which is non-miscible with the selected lipophilic phase.
20

In a preferred aspect, it has been found that in compositions of the present invention, the use of a mono- or polyhydroxyalcohol co-surfactant, such as ethanol, butanol or propylene glycol, as the major component of the hydrophilic phase may be avoided. This has the advantage of not only mitigating the stability and processing difficulties associated with the use of such but also reducing the concomitant stomach and duodenum irritation. Accordingly, the hydrophilic phase of compositions of the present invention may be essentially aqueous and comprise less than 10%, preferably less than 5% and more preferably less than 2% by weight of the phase of an alcohol.
25

It will be readily appreciated by the skilled person that not all blends of a fatty acid triglyceride, low and high HLB surfactants and hydrophilic phase will yield stable, self-emulsifying microemulsions within the scope of the present invention. Appropriate ratios may, however, be readily determined by the skilled man with the aid of a phase diagram such as that illustrated in Fig. 1. As the system comprises four components viz a fatty
30 acyl triglyceride (oil), a low HLB surfactant, a high HLB surfactant and an aqueous/hydrophilic phase, a pseudo-ternary phase diagram is employed. In this, the ratio of two components such as the oil and the low HLB surfactant is kept constant so

that there are only three variables, each of which can then be represented by one side of the triangle. Thus, in Fig. 1, (1) represents the mixture of the oil and the low HLB surfactant, at a fixed ratio X, (2) the hydrophilic (aqueous) phase and (3) the high HLB surfactant. By way of example, the point "A" represents a mixture 50% oil plus low HLB surfactant, 20% aqueous phase and 30% high HLB surfactant.

The regions of the phase diagram in which microemulsions according to the present invention exist may be determined by titrating a mixture of the oil and low HLB surfactant (in a fixed ratio) against the high HLB surfactant and the hydrophilic phase, noting points of phase separation, turbidity and transparency. Clear, transparent formulations are indicative of the formation of a stable microemulsion. Liquid and gel formulations may be obtained at room temperature according to the specific nature of the components employed.

Once stable transparent systems are obtained, simple tests, such as dye solubilization, dispersibility in water and conductivity measurements may be used to determine whether the microemulsion is an (o/w)- or a (w/o)-type. A water-soluble dye will disperse in an (o/w) microemulsion while it will remain in its original form in a (w/o) microemulsion. Likewise, (o/w) microemulsions are generally dispersible in water whereas (w/o) microemulsions are generally not. In addition, (o/w) microemulsions conduct electricity whereas (w/o) do not. The isotropic nature of the system may be confirmed by examination thereof under polarised light. The microemulsions being micellar in nature are isotropic and therefore non-birefringent when examined under polarised light.

25

From this phase diagram, appropriate percentages may then be read off. The process may then be repeated for other ratios of oil to low HLB surfactant so that an overall picture may be obtained.

30

A representative pseudo-ternary phase diagram of a system containing in the lipophilic phase an interesterified medium-chain triglyceride oil (CAPTEX 810A) and a long-chain fatty acyl monoglyceride (ARLACEL 186, low HLB surfactant) (in the ratio 3:1), high HLB surfactant (TWEEN 80) and saline is shown as Fig 2. The mixture of oil plus the low HLB surfactant is indicated as component (1), saline as component (2) and the high HLB surfactant as component (3). These systems produce a wide range of clear, transparent microemulsions which are shown in the phase diagram as the microemulsion field (shaded areas) which field may be usefully be sub-divided into regions (A), (B) and (C).

This sub-division is based primarily on differences in conductance, viscosity and dilutability in the presence of excess water (at least 5-fold). Both the viscosity and conductance increase from region (A) to (C), with major changes observed between (B) 5 and (C). In the presence of excess of the dispersed phase (saline or water), microemulsions of regions (A) and (B) are inverted to turbid (o/w) emulsions. In contrast, microemulsions from region (C) remains clear up on dilution.

The calculated final HLB values for the blend of low and high HLB surfactants in 10 the regions (A), (B) and (C) are 7 to 11, 11 to 13 and 13 to 15, respectively.

Microemulsions within the scope of the present invention are those falling within regions (A), (B) and (C) of the pseudo-ternary phase diagram.

15 Accordingly, in a further aspect the present invention provides compositions which form stable, self-emulsifying (w/o) microemulsions as hereinbefore defined in which the relative proportions of the various components lie within regions (A), (B) and (C), preferably (A) and (B), more preferably (A), of the pseudo-ternary phase diagrams as described herein, such as in Figure 2 for instance.

20 In general, in the representative system, stable, clear, transparent liquid microemulsions were obtained when the oil plus low HLB surfactant was present in the range from about 40% to less than 100%, the high HLB surfactant less than 50% and the water less than 20% (w/w) of the microemulsion.

25 By this process of constructing a representative range of phase diagrams, it is possible to determine appropriate quantities of the various components which will lead to stable, self-emulsifying microemulsions falling within the present invention.

30 Suitably, the lipophilic phase comprising fatty acyl interesterified triglyceride and the low HLB surfactant together comprise from about 8 to about 95%, preferably about 10 to about 90%, more preferably about 40 to about 90%, most preferably about 60 to about 90% (w/w) of the microemulsion. The fatty acyl triglyceride and the low HLB surfactant may be combined and mixed at various ratios. Useful (w/o) microemulsions of relatively 35 low viscosity may be obtained when the ratio of fatty acyl triglyceride to low HLB surfactant is in the range of about 5:1 to about 1.5:1, preferably about 4:1 to about 2:1. It is found that as the ratio of fatty acyl triglyceride to low HLB surfactant is increased towards 5:1, region (C) of the microemulsion existence field becomes increasingly

predominant. Suitably, microemulsions of the present invention comprise in the lipophilic phase at least 50% of medium-chain components. Preferably, the ratio of medium- to long-chain components is from about 9:1 to 1:1, more preferably from about 6:1 to 1:1, most preferably about 4:1 to 1:1.

5

Suitably, the high HLB surfactant is present in the range of about 5 to about 75%, preferably about 5 to about 50%, more preferably from about 7.5 to about 30% (w/w) of the microemulsion.

10

Suitably the hydrophilic phase comprises from just greater than 0 to about 40%, preferably from about 0.1 to 20%, more preferably from about 0.1 to 10% and most preferably from about 1 to 5% (w/w) of the microemulsion.

15

It will be readily appreciated by the skilled person that, in general, an increase in the relative amount of high HLB surfactant will have to be matched by an increase in the relative amount of hydrophilic phase.

20

In microemulsions of the present invention, the lipophilic phase comprises preferably about 10-90%, more preferably 40 to 90%, most preferably 60 to 90%, the high HLB surfactant preferably from about 5 to 75%, more preferably from 5 to 50%, most preferably 7.5 to 30% and the hydrophilic phase preferably less than 40%, more preferably less than 10% and most preferably less than 5% (w/w) of the microemulsion. Within such microemulsions, the ratio of fatty acyl triglyceride to low HLB surfactant is preferably between 4:1 and 2:1.

25

The microemulsions of the present invention are substantially non-opaque, that is they are transparent or opalescent when viewed by optical microscopic means. In their undisturbed state, they are optically isotropic (non-birefringent) when examined under polarized light. They exhibit excellent stability at low and ambient temperatures, without phase separation, clouding or precipitation, even over prolonged periods of time. The formulations may be stored in a stable form at various temperatures, such as at 4°C, ambient temperature, 37°C and at 50°C, preferably at 4°C or ambient temperatures.

30

Peptide-containing microemulsions of the present invention exhibit a similar stability (shelf life) profile to that of the corresponding peptide-free microemulsions. Stable (w/o) microemulsions may be formed when the pH of the aqueous phase varies from a pH of approximately 3 to about 10, a property that can be beneficial for drugs exhibiting higher solubility at low or high pH. The microemulsions are of varying viscosity, with

formulations which are mobile liquids or gels at ambient temperature. Microemulsions with a relatively higher amount of a high HLB surfactant such as TWEEN 80 tend to be more viscous due to the greater viscosity of this material.

5 Preferably, the diameter of droplets or particles of the microemulsions of the present invention, measured, for instance, as the number-average diameter by laser light scattering techniques, is less than 150 nm, more preferably less than 100 nm, yet more preferably less than 50 nm and most preferably in the range 5 to 35 nm.

10 The various phases may optionally contain further ingredients, such as, but not limited to:

- i) lipids, such as phospholipids, in particular lecithins, such as soya bean lecithins, egg lecithin or egg phosphatide, cholesterol or long-chain fatty acids such as oleic acid;
- 15 ii) antioxidants such as n-propyl gallate, butylated hydroxyanisole (BHA) and mixed isomers thereof, d-a-tocopherol and mixed isomers thereof, ascorbic acid, propylparaben, methylparaben and citric acid (monohydrate), for instance in amounts less than 3, preferably less than 1% (w/w);
- iii) bile salts, for instance as their alkali metal salts, such as sodium taurocholate;
- 20 iv) stabilizers, such as hydroxypropyl cellulose, for instance in amounts less than 3, preferably less than 1% (w/w);
- v) antimicrobials, such as benzoic acid (sodium salt);
- vi) dioctylsuccinate, di-octylsodium sulfosuccinate or sodium lauryl sulfate;
- vii) propylene glycol mono-and di-fatty acid esters, such as propylene glycol dicaprylate, dilaurate, hydroxystearate, isostearate, laurate, ricinolate, etc., of which the propylene glycol caprylic/capric acid diesters commercially known as Miglyol 840 and Imwitor 408 are especially preferred; and
- viii) protease inhibitors such as aprotinin.

30 The microemulsions of the present invention form spontaneously or substantially spontaneously when their components are brought into contact, that is without the application of substantial energy supply, for instance in the absence of high shear energy such as imparted by homogenization and/or microfluidization or other mechanical agitation. Accordingly the microemulsions may be readily prepared by the simple process
35 of admixing appropriate quantities, with gentle hand mixing or stirring if necessary to ensure thorough mixing. Preferably, the drug is dissolved in the hydrophilic phase, either directly or by dilution of a stock solution thereof and this may then be added to a pre-mixed combination of the oil and the low HLB surfactant with mixing, followed by the

high HLB surfactant or *vice versa*. Alternatively, a drug-free microemulsion may be initially prepared by admixing the oil, the low HLB surfactant, the high HLB surfactant and drug-free hydrophilic phase; to which may then be added further hydrophilic phase in which the drug is dissolved. While higher temperatures (40-60°C) may be needed to 5 solubilize all components during the preparation of the microemulsion, the preferred systems may be formulated at room temperature. Formulation at ambient temperature is particularly advantageous for thermolabile active ingredients such as peptides.

10 The pharmaceutical compositions of the present invention comprise a therapeutic agent and are intended for use in therapy, for administration to animals, including man.

Accordingly, in a further aspect, the present invention provides a method of treatment which comprises administering an effective amount of a pharmaceutical composition as hereinbefore defined to a patient in need thereof.

15 It will be recognized by the skilled man that the amount of drug required for therapeutic effect will vary with the drug chosen, the nature and severity of the condition and the animal undergoing treatment and is ultimately at the discretion of the physician. Furthermore, the optimal quantity and spacing of individual dosages of a drug will be 20 determined by the nature and extent of the condition being treated, the form, route and site of administration, the particular patient being treated and that such optima can be determined by conventional techniques. It will also be appreciated that the optimal course of treatment, that is, the number of doses given, may be readily ascertained using conventional course of treatment determination tests.

25 In a further aspect, the present invention provides for the use of a fatty acyl triglyceride, a low HLB surfactant, a high HLB surfactant, a therapeutic agent and a hydrophilic phase as hereinbefore defined in the manufacture of a medicament.

30 Pharmaceutical compositions of the present invention may be used for oral, topical, rectal, intra-vaginal or other forms of systemic administration and accordingly will be presented in forms suitable for such. Thus for instance, pharmaceutical compositions intended for oral administration may be presented in soft gelatin capsules while the viscosity characteristics of some of the pharmaceutical compositions make them suitable 35 for direct topical application. Compositions suitable for oral or topical administration are especially preferred.

- The microemulsion compositions of the present invention without a drug are novel and useful as precursors to drug-containing microemulsions. Accordingly, in a further aspect, the present invention provides a composition comprising (a) a lipophilic phase having an oil which comprises an interesterified triglyceride and a low HLB surfactant
- 5 which is a medium- or a long-chain fatty acyl mono- and/or diglyceride, a sorbitan long-chain fatty acid ester or a mixture thereof; (b) a high HLB surfactant and (c) an aqueous hydrophilic phase which on admixing form a stable, self-emulsifying, water-in-oil (w/o) microemulsion.
- 10 The invention will now be illustrated by, but not limited to, the following descriptions (drug-free compositions) and examples (drug-containing compositions), with reference to figures 1 to 8 as hereinbefore described above,

DESCRIPTIONS

15

Description 1 - Phase Diagrams for Representative Compositions

Pseudo-ternary phase diagrams were constructed for the following representative systems comprising in the lipophilic phase:

20

No.	Fatty Acid Triglyceride	Low HLB Surfactant	Ratio	Fig.
1	CAPTEX 810A	ARLACEL 186	3:1	2
2	CAPTEX 810A	CAPMUL MCM	3:1	3
3	CAPTEX 910A	ARLACEL 186	3:1	4
4	CAPTEX 910A	CAPMUL MCM	3:1	5
5	CAPTEX 910B	ARLACEL 186	3:1	6
6	CAPTEX 910B	CAPMUL MCM	3:1	7
7	CAPTEX 910C	ARLACEL 186	3:1	8
8	CAPTEX 910C	CAPMUL MCM	3:1	9

in combination with TWEEN 80 as the high HLB surfactant and saline as the hydrophilic phase.

25

The region of the phase diagram in which microemulsions were formed was determined by titrating a mixture of the oil and low HLB surfactant (in a fixed ratio) against the high HLB surfactant and the aqueous phase, noting points of phase separation, turbidity and transparency.

The resultant phase diagrams are shown as Figures 2 to 9. A wide range of clear, transparent, liquid (w/o) microemulsions as shown by regions (A), (B) and (C) were available. These are stable at room temperature and at 37°C.

5

From these, the skilled person will readily appreciate that the microemulsion existence regions for other systems may be readily determined by focussing on the ratios defined by regions (A), (B) and (C) rather than having to repeat the whole process and look at relative amounts well removed from these regions.

10

EXAMPLES

Examples of microemulsions containing a therapeutic agent which was either GHRP or RGD were prepared according to the standard procedure outlined below and with the

15 proportions given in the following table:

Example	Drug	Drug conc. mg/g form.	oil ^a & low HLB ^b surfactant %(w/w)	high HLB surf. ^c %(w/w)	aqueous phased ^d %(w/w)
1	GHRP ^e	1.32	60	35	5
2	GHRP	0.92	86.5	10	3.5
3	RGD ^f	4.65	45	45	10
4	RGD	2.32	75	20	5
5	GHRP	0.68	82	15	3
6	RGD	1.4	80	17	3
7	GHRP	0.9	75	21	4
8	RGD	2.32	60	35	5
9	GHRP	1.7	62	30.5	7.5
10	RGD	1.16	85	12.5	2.5
11	GHRP	3.35	20	65.2	14.8

Footnotes to table

^aexamples 1 to 4: CAPTEX 810A; examples 5 to 8: CAPTEX 910A; examples 9 and 10:

20 CAPTEX 910B; example 11: CAPTEX 910C.

^bARLACEL 186 (examples 2, 4, 7, 8 - 10); CAPMUL MCM (examples 1, 3, 5, 6, 11).

^cTWEEN 80

^d isotonic soln containing acetic acid and sodium chloride at pH 5.0 (examples 1, 2, 5, 7, 9 and 11); saline (examples 3, 4, 6, 8 and 10).

^e His-D-Trp-Ala-Trp-D-Phe-Lys-NH₂; and

^f cyclo(S,S)-(2-mercaptophenyl)benzoyl-(N³-methyl)-Arg-Gly-Asp-(2-mercaptophenyl)amide.

5

Such microemulsions were generally formulated by initially preparing the drug-containing hydrophilic phase, either by dissolving the appropriate amount of drug in the appropriate amount of saline solution or, more preferably, using a stock solution which was then further diluted if so required, with vortex stirring if necessary to obtain complete 10 dissolution. The hydrophilic phase containing the drug was then added to the appropriate amounts (by weight) of a mixture of the oil and the low HLB surfactant, to which was then added the high HLB surfactant, with gentle stirring (magnetic hot plate stirrer). Alternatively, the hydrophilic phase containing the drug was added to the high HLB 15 surfactant and following upon complete mixing, this was added to the oil plus low HLB surfactant mixture. If necessary, the drug-containing microemulsion was then diluted with the corresponding drug-free microemulsion to adjust the concentration of the drug.

METHODS OF TREATMENT

20 The formulations of the present invention are tested for GI irritation assessment without an active ingredient by the following method:

Oral Dosing in Rats/GI Irritation Assessment

Suitable rats for use in this assessment are male Sprague-Dawley (Caesarian Delivery - Virus Antibody Free; Charles River Laboratories). The rats are fasted overnight the day 25 before the experiment. Dosing with the microemulsion at the desired dose is done by gavage at a volume not exceeding 10 ml/kg. Upon termination of the experiment animals are euthanized with asphyxiation using carbon dioxide and exsanguinated. Abdominal incisions are then performed and gross observations of the gastric and duodenal mucosa are made at naked eyes and under a microscope (Nikon model SMZ-10 binocular microscope).

30 One aspect of the present invention are the formulations of w/o self-emulsifying microemulsions with or without peptide which produce little, if any, damage along the GI tract upon oral administration. The present formulations of Examples 1 to 8, for instance are given orally by gavage (preferably at three rats per formulation). After 24 hrs the animals are exsanguinated and upon abdominal incisions are examined both by naked eye and under 35 the microscope. The mucosal surface of both the stomach and duodenum of the animals that received microemulsions containing CAPTEX/CAPMUL or CAPTEX/ARLACEL are examined to see if they are free of any lesions at naked eye.

Oral Bioavailability of an RGD Peptide in Rats:

In the procedure described below microemulsions formulated as described above and containing, for instance, 3mg of peptide per gr of microemulsion are tested in the following manner for oral bioavailability.

5 a) Intravenous (iv) administration of peptide in saline

Fasted rats are given an intraperitoneal (i.p.) injection and surgically fitted with femoral artery catheters. Rats are allowed to recover from the surgery for 1 day. Catheterized rats
10 are fasted for 18 hr prior to the experiment. Each rat receives 3mg of peptide by lateral tail-vein administration from a solution prepared as follows:

15 10.84 mg peptide q.s. to 8ml with 0.9% saline solution. Blood samples of 0.5ml aliquots are collected at 0, 1, 3, 5, 10, 15, 30, 45, 60, 90, 120, 150 and 180 minutes. The 0 min. sample is taken 15 min prior to administration of the dose. Plasma is removed from the whole blood by centrifugation at 16000Xg for 5 min, and then plasma is stored at -20°C in 250µl aliquots per sample. The blood pellet is reconstituted with heparinized saline and returned to the appropriate rat via catheter. After the experiment, rats were euthanized with iv administration of pentobarbital.

20 b) Intraduodenal (i.d.) administration of peptide in microemulsion

Fasted rats are given an i.p. injection of anesthesia cocktail and surgically fitted with jugular and duodenal catheters. Rats are allowed to recover from the surgery for 4-5 days. Catheterized rats are fasted 18-20 hrs. prior to the experiment. Each rat receives 10mg of peptide in either microemulsion or saline solution. Blood samples of 0.5ml aliquots are
25 collected via jugular catheter in heparinized eppendorf tubes at 0, 10, 30, 60, 120, 180, 240 and 1440 minutes. The 0 min sample is taken 15 min prior to administration of the dose by duodenal catheter. Plasma is collected for analysis and the blood returned to rats as described in the i.v. administration (part a) above. After 1440 min, rats are euthanized by iv administration of pentobarbital, exsanguinated and the GI tract removed for gross
30 observation.

c) Analysis of peptide plasma concentration

Standards are placed before and after the sample for HPLC analysis. A 50µl aliquot for 0-200 ng peptide, 25µl aliquot for 1000-2000 ng peptide, 15µl aliquot for 10,000 ng peptide and a 50µl aliquot of each sample is analyzed by post-column fluorescence detection.

35 Fluorescence chromatography data is collected and integrated using a Nelson Chromatography Data System. The peak area ration (Y) and peptide standard concentration (X) are used to determine the slope of a line which is forced through the origin from the

equation: slope = (sum of X*Y)/(Sum of X²). The slope represents the relationship between peak area ratio and peptide plasma concentration for the samples.

d) Calculation of Bioavailability

5 First, the area under the plasma concentration curve (AUC) from 0 to 240 minutes is determined for each rat. For id administration, percentage bioavailability is determined for each animal by the following equation with the average AUC from iv administration:
[(AUC_{id}/AUC_{iv})*(dose_{iv}/dose_{id})] * [100].

10 The oral bioavailability data for the RGD peptide in rats after intraduodenal administration of a microemulsion containing the above formulations incorporating a fibrinogen receptor antagonist of a peptide dose may then be obtained in the above noted manner.

15 When applicable, the formulations of the present invention are tested for *in vivo* activity. As one of the active ingredients utilized herein is a fibrinogen receptor antagonist a platelet aggregation assay is employed to determine pharmacological activity of the peptide from microemulsions. These studies are carried out as shown below.

20 Oral Dosing in Dogs/Platelet Aggregation Assay :

Dogs used in this assay are male Mongrels (i.e. from mixed breeds). The dog(s) are fasted overnight the day before the experiment. The cephalic vein of choice is prepared for the indwelling catheter in the following way: the area is first shaved and cleaned with a gauze soaked in 70% alcohol. An indwelling catheter is placed in the caphalic vein and attached to a
25 luer lock adapter filled with 3.8% sodium citrate. The catheter is securely taped down. When a blood sample is withdrawn, a 0.3 ml of blood is withdrawn into a separate 1 cc syringe before the actual sample so that dilution of the blood sample from the sodium citrate contained in the luer lock adapter is avoided. Then 2.7 ml of blood are drawn in a 3 cc
30 syringe and placed in a Venoject vacuum tube containing 0.3 ml of 3.8% sodium citrate and labelled with the appropriate time point. The tube containing the blood sample in 3.8% sodium citrate is gently inverted few times to mix components and then 1 ml is withdrawn for the whole blood aggregation assay. The rest of the blood sample is transferred to an eppendorff tube and upon centrifugation the supernatant plasma is removed and transferred to a new tube which wis then frozen for subsequent HPLC analysis to determine peptide
35 content.

Just after the zero time point blood sample is withdrawn, an appropriate dose of microemulsion with or without peptide is administered orally to the dog using a size 12 gelatin capsule.

- The blood samples are then assayed for platelet aggregation inhibition using the
- 5 Chromo-Log whole blood aggregometer. The instrument is warmed to 37°C before samples are run and the probe is cleaned with distilled water and a soft brush. The probe is attached to the aggregometer and placed in a cuvette of saline solution and warmed in a side cuvette well in the aggregometer. For the actual assay, 1 ml of the 2.7 ml of blood sample mixed with the 0.3 ml 3.8% sodium citrate contained in the Venoject vacuum tube is added to a
- 10 cuvette and placed in the aggregometer well. A stir bar is placed in the cuvette and set at 900 rpm. The probe is placed firmly into the test cuvette and the lid is shut. Baselines, zero and calibration are set. Calibration is set equal to 20 = 5 ohms. The stirring cuvette is permitted to settle for five minutes at which point 5 µl of collagen is added to the whole blood that is being stirred to yield to a 5 µg/ml final solution in the cuvette.
- 15 The reaction is monitored for two minutes once the slope change reaches the baseline of the collagen addition, calculating the change in ohms per minute using the slope of the two minutes. The change in ohms per minute is calculated as a % of the control. The control value is determined by the average of the -15 and the 0 time points. After each use the probe is removed and cleaned with distilled water and wiped with a soft cloth and brush.
- 20

Discussion and Conclusion:

- A dog is considered a good model to assess the pharmacological effect of one class of peptides of interest herein, the RGD containing fibrinogen receptor antagonists. Experiments are conducted as described above, with a peptide dose of 3 mg/kg or microemulsion dose of
- 25 0.5 ml/kg. Control experiments where the peptide is given orally in a saline solution are independently carried out earlier and serve as a useful comparison to the effects seen with the microemulsion-formulated peptide.

- As one of the active ingredients utilized herein is a Growth Hormone Releasing Peptide
- 30 the appropriate assay for in vivo activity is determined as shown below.

In Vivo Testing of GHRP-Containing Microemulsion:

- A microemulsion with a composition (w/w) in accordance with Formulas 1 to 8 above are made. Upon preparation, they are further stored in a stable form at ambient
- 35 temperature for approximately 48 hrs before the in vivo evaluation. A control solution of a GHRP peptide, His-D-Trp-Ala-Trp-D-Phe-Lys-NH₂, in saline at 1.5 mg/ml is also prepared.

Dosing is done by single intraduodenal administration of GHRP at 3 mg/kg in male rats in saline solution (control) and in the aforementioned microemulsion using 3 rats in each case. Prior to actual sampling and dosing, each rat is anesthetized with Pentobarbital at 50 mg/kg i.p. diluted with saline to a final volume of 1 ml. The rats stay anesthetized for the entire experiment. Dosing is achieved in the following way: a small incision 2-3 cm long is made on the abdominal midline, and then a purse-string suture is placed on the duodenal muscle. A small hole is made in the center of the purse-string suture in which a blunt 23 G stub needle attached to a tuberculin syringe is inserted to deliver the dose. Upon completion of dosing, the purse-string is tied to close the opening. The incision is closed with wound clips. A 0.2 ml blood sample is obtained via jugular catheter at the following intervals: -15, 0, 5, 10, 15, 30, 45, 60, 90, and 120 minutes. Blood samples are stored on ice and subsequently analyzed for Growth Hormone by an RIA method.

Analysis of the samples generated from the experiment mentioned above need to have determined the pharmacological activity of GHRP. Positive data will indicate that Growth Hormone Releasing Peptide is orally active from the microemulsion formulation of the present invention. However, blood levels and actual bioavailability need to be correlated to observed pharmacological activity.

The amount of active ingredient required for therapeutic systemic administration will, of course, vary with the compound chosen, the nature and severity of the condition, and the mammal, including humans, undergoing treatment, and is ultimately at the discretion of the physician.

Ultimately, the present invention also includes a method of treatment which comprises administering an effective amount of a pharmaceutical composition as defined herein to a patient in need thereof. Preferably, the therapeutic agent is selected from fibrinogen receptor antagonist peptide, Growth Hormone Releasing Peptide, vasopressin, elcatonin, calcitonin, calcitonin-gene related peptide, porcine somatostatin, or insulin. The disease states and uses of each of the aforementioned therapeutic agents is well known to those skilled in the art and for a number of the agents already cross referenced to their respective patents. For instance, use as platelet aggregation inhibitors, growth promoters, for osteoporosis, and diabetes.

The above description fully discloses the invention including preferred embodiments thereof. Modifications and improvements of the embodiments specifically disclosed herein are within the scope of the following claims. Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present

invention to its fullest extent. Therefore the Examples herein are to be construed as merely illustrative and not a limitation of the scope of the present invention in any way. The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows.

What is claimed is :

1. A pharmaceutical composition comprising:
 - (a) a lipophilic phase having an oil which comprises an interesterified triglyceride and a low HLB surfactant wherein the low HLB surfactant is selected from a medium fatty acyl monoglyceride, a long chain fatty acyl mono-glyceride, a long-chain fatty acyl di-glyceride, a medium chain fatty acyl di-glyceride, a sorbitan long-chain fatty acid ester or mixtures thereof;
 - (b) a high HLB surfactant;
- 10 (c) an aqueous hydrophilic phase; and
- (d) a water-soluble therapeutic agent;
which on admixing form a stable, self-emulsifying, water-in-oil (w/o) microemulsion.
2. A composition as claimed in claim 1 in which the interesterified triglycerides comprise medium-chain fatty acyl moieties which are caprylic or capric acids or caprylic and capric acids, and the long-chain fatty acyl moieties are oleic or linoleic acids, or are oleic and linoleic acids.
- 15 3. A composition as claimed in claim 2 in which the medium-chain fatty acyl moieties comprise from about 30 to 90% and the long-chain fatty acyl moieties comprise from about 10 to 50% by weight of the fatty acyl moieties.
- 20 4. A composition as claimed in claim 3 in which the medium-chain fatty acyl moieties comprise a majority of caprylic acid moieties.
- 25 5. A composition as claimed in claim 1 in which the low HLB surfactant is a medium or long chain fatty acyl monoglyceride or a medium or long chain fatty acyl di-glyceride or is a mixture thereof.
- 30 6. A composition as claimed in claim 5 in which the low HLB surfactant comprises medium chain fatty acyl caprylic acid/capric acid mono- and di-glycerides.
- 35 7. A composition as claimed in claim 1 in which high HLB surfactant is a non-ionic surfactant selected from (a) polyoxyethylene fatty acid esters, (b) polyoxyethylenesorbitan fatty acid esters, (c) polyoxyethylene glycol long-chain alkyl ethers and (d) polyoxyethylene glycol long-chain alkyl esters.
8. A composition as claimed in claim 1 in which the therapeutic agent is a peptide.

9. A composition as claimed in claim 1 in which the peptide has a molecular weight of between 100 and 10,000.
- 5 10. A composition as claimed in claim 1 in which the peptide has from 2 to 35 amino acid residues.
11. A composition as claimed in claim 8 in which the peptide is selected from a fibrinogen receptor antagonist peptide, a growth hormone releasing peptide, a
- 10 vasopressin, a calcitonin or an insulin.
12. A composition as claimed in claim 1 in which the relative proportions of the lipophilic phase, the high HLB surfactant and the aqueous phase lie within regions (A), (B) and (C) of the pseudo-ternary phase diagrams of Figures 2 to 8.
- 15 13. A composition as claimed in claim 1 in which the fatty acyl interesterified triglyceride and the low HLB surfactant together comprise from about 8 to about 95% (w/w) of the microemulsion.
- 20 14. A composition as claimed in claim 13 in which the ratio of fatty acyl triglyceride to low HLB surfactant is in the range of 5:1 to 1.5:1.
15. A composition as claimed in claim 1 in which the ratio of medium- to long-chain components is from 9:1 to 1:1.
- 25 16. A composition as claimed in claim 1 in which the lipophilic phase comprises from 10-90%, the high HLB surfactant from 5 to 75% and the hydrophilic phase less than 40% (w/w) of the microemulsion and in which the ratio of fatty acyl triglyceride to low HLB surfactant is between 4:1 and 2:1.
- 30 17. The microemulsion according to claim 1 adapted for oral delivery or topical application.
18. A composition comprising:
 - 35 (a) a lipophilic phase having an oil which comprises an interesterified triglyceride and a low HLB surfactant which is a medium- or a long-chain fatty acyl mono- and/or diglyceride, a sorbitan long-chain fatty acid ester or a mixture thereof;
 - (b) a high HLB surfactant; and

(c) an aqueous hydrophilic phase;

which on admixing form a stable, self-emulsifying, water-in-oil (w/o) microemulsion.

19. The microemulsion according to claim 18 which further provides for oral

5 bioavailability enhancement of a therapeutic agent in a mammal.

20. A process for production of a pharmaceutical composition which process comprises:

(a) admixing

10 (i) a lipophilic phase having an oil which comprises an interesterified triglyceride and a low HLB surfactant wherein the low HLB surfactant is selected from a medium fatty acyl monoglyceride, a long chain fatty acyl mono-glyceride, a long-chain fatty acyl di-glyceride, a medium chain fatty acyl di-glyceride, a sorbitan long-chain fatty acid ester or mixtures thereof; with

15 (ii) a high HLB surfactant; and

(iii) an aqueous hydrophilic phase; and

(iv) a water-soluble therapeutic agent; and

(b) forming a stable, self-emulsifying, water-in-oil (w/o) microemulsion.

1/5

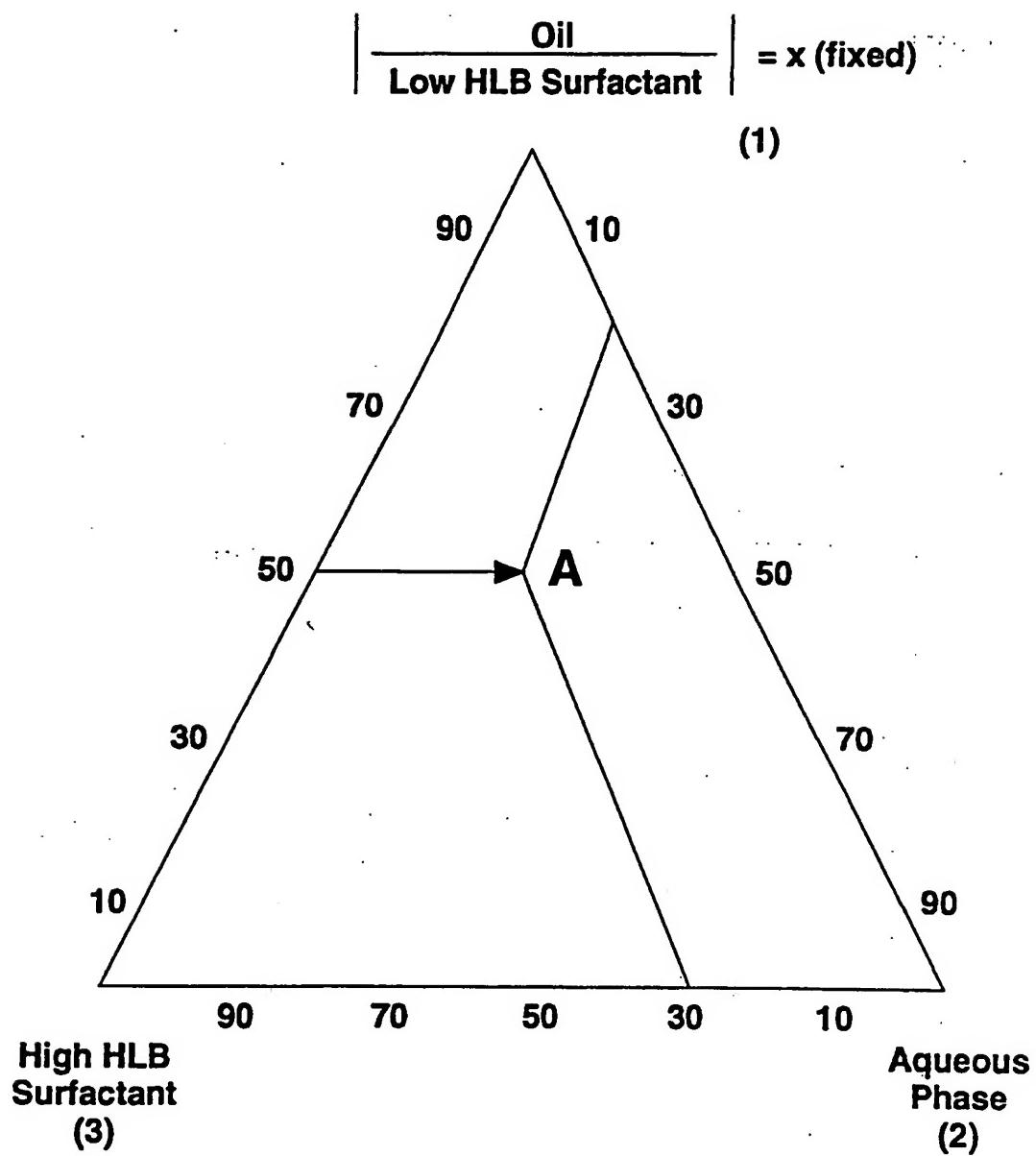
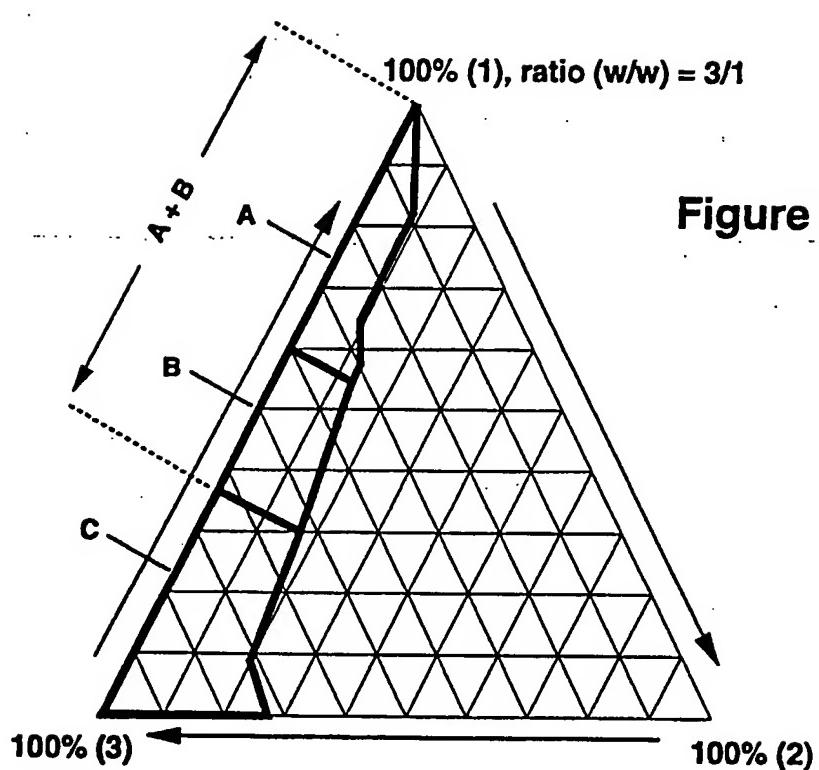
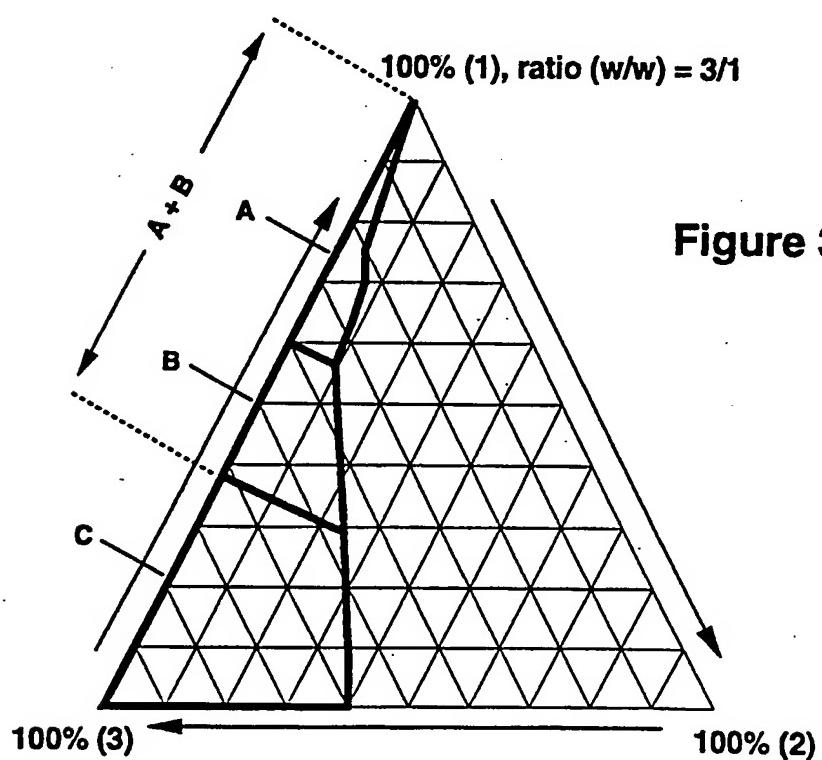
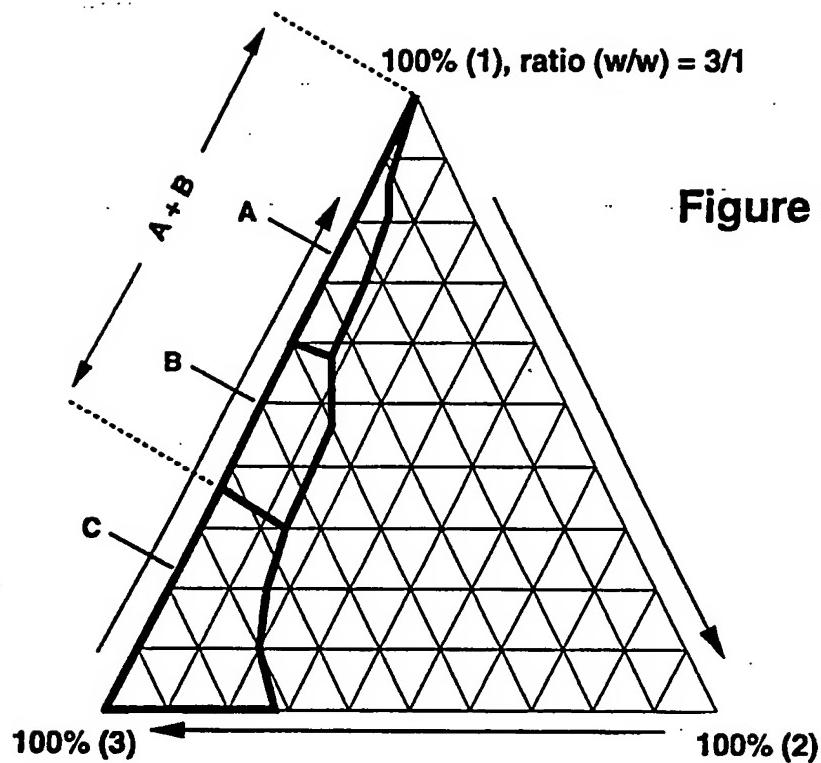
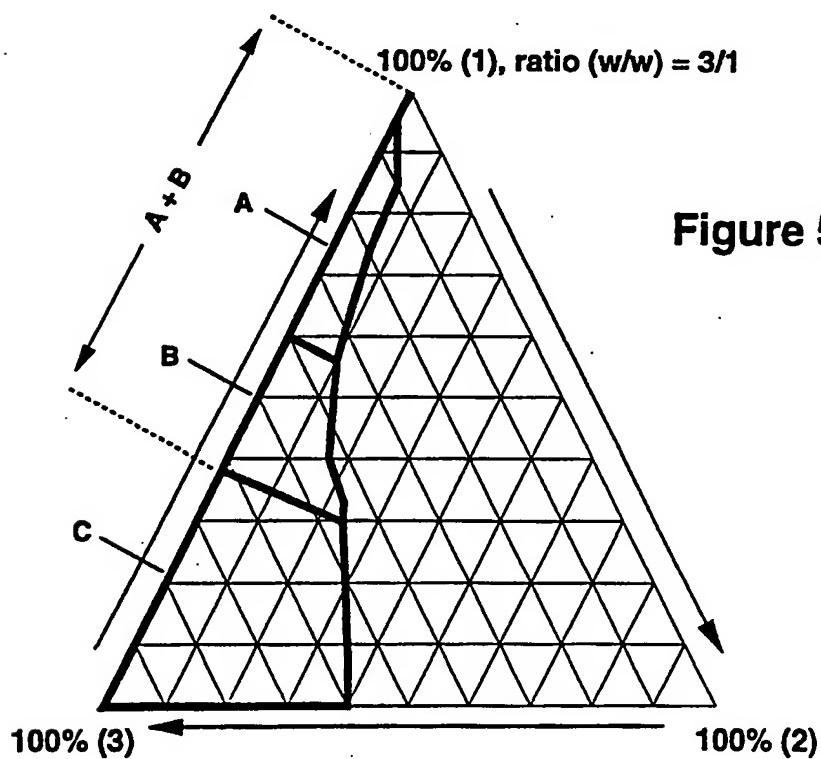


Figure 1

2/5

**Figure 2****Figure 3**

3/5

**Figure 4****Figure 5**

4/5

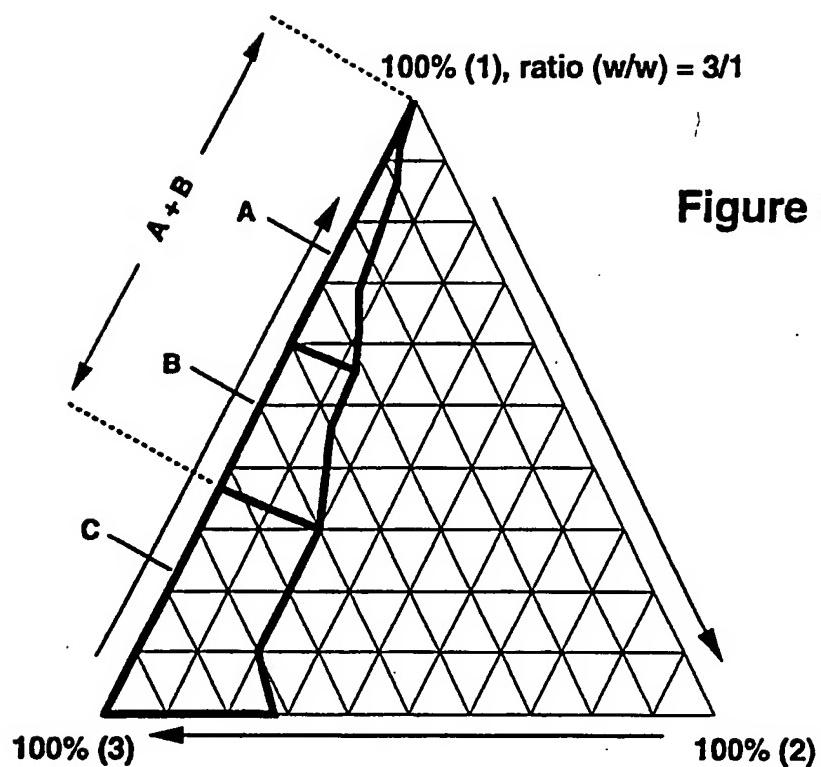


Figure 6

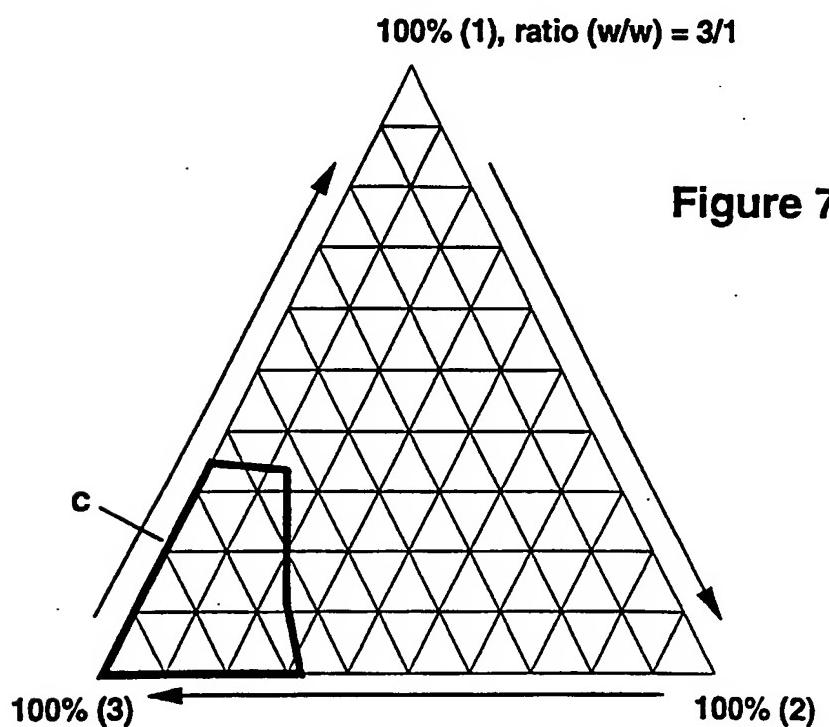
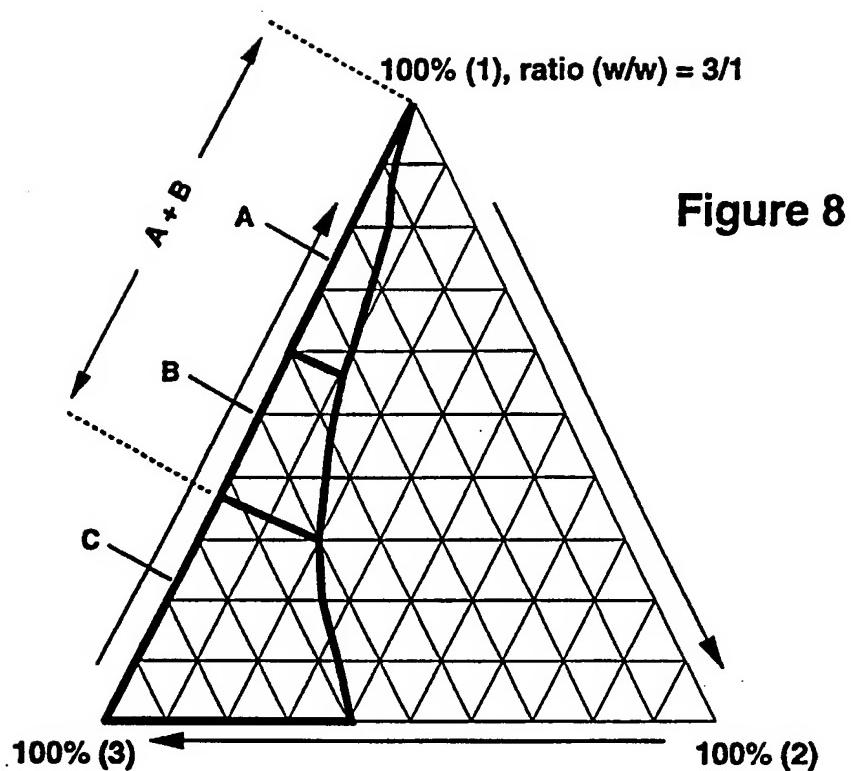
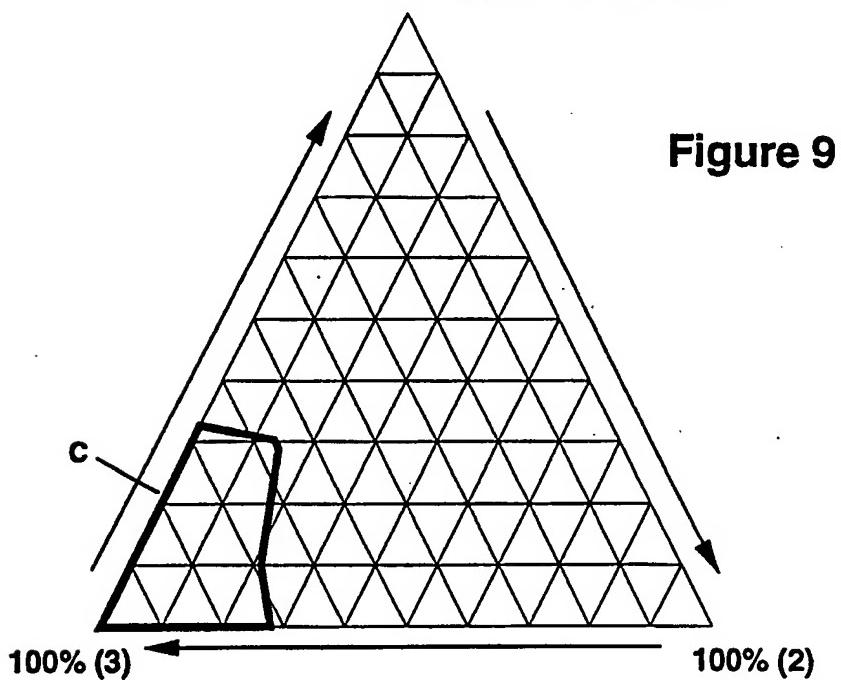


Figure 7

5/5



100% (1), ratio (w/w) = 3/1



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US93/09915

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 37/00, 37/02, 37/26
 US CL :514/02, 03, 12, 17, 18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/02, 03, 12, 17, 18

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CAS ONLINE, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US, 3,876,765 (Choay) 08 April 1975, see entire document.	1-20

<input type="checkbox"/>	Further documents are listed in the continuation of Box C.	<input type="checkbox"/>	See patent family annex.
A	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"P"	document referring to an oral disclosure, use, exhibition or other means		
	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
11 JANUARY 1994	31 JAN 1994
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer <i>Jill Warden for</i> CAROL A. SALATA, PH.D.
Facsimile No. NOT APPLICABLE	Telephone No. (703) 308-0196